Journal of the University of Chemical Technology and Metallurgy, 47, 1, 2012, 63-68

PRETREATMENTS EFFECT OF SORGHUM (*Bicolor (L.) Moench)* AND MILLET (*Pennisetum Glaucum)* FLOURS ON THE IN VITRO STARCH DIGESTIBILITY

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Received 20 January 2009
Accepted 10 January 2012

ABSTRACT

The starch digestibility of sorghum and millet flours was studied using 1 % suspensions of sorghum and millet flours. The two suspensions were exposed to an enzymatic hydrolysis by glucoamylase. After two hours the digested starch was measured. At these conditions the starch digestibility of both flours is approximately 36.4 % for sorghum and 36.8 % for millet. After that the two flours were pretreated with hexane to remove some lipids. The starch digestibility was estimated as 36.6% for sorghum and 36.2% for millet. Then some proteins were removed using a pretreated flours with the pepsin enzyme and the starch digestibility was estimated as 56.6 % for millet and 55.4 % for sorghum. After that the two suspensions were treated with hexane and pepsin. An increase of the starch digestibility was observed in both samples, 87.6 % for sorghum and 84.5 % for millet. Finally, after starch isolation, the starch digestibility increased to 89.5 % for sorghum and 89.7 % for millet.

Keywords: glucoamylase, sorghum, millet, flours, digestibility, starch, pretreatments.

INTRODUCTION

Sorghum and millet are an important source of dietary energy and the starch represents the major source in these cereals that are well adapted to African and Asian semi-arid and subtropical agronomic conditions [1]. Amylases are the enzymes that hydrolyse starch contained in foods. The rate of starch digestibility can be estimated in vitro [2]. The starch digestibility of both cereals depends on the presence of reducing agents such as proteins, lipids, fiber and the chemical and physical characteristics of cereal starch [3]. The effect of amylase-lipid complex on the gelatinization of starch [4], as well as the reducing impact of proteins on digesting enzymes of starch, reduce the contact between enzyme and substrats. The sorghum grain displays a low digestibility of starch due to the endosperm proteins [5] and the presence of fibres, polyphenol compounds, resistant starch and Kafirins protein [6,7]. Zhang and Hamaker demonstrated the effect of protein of cooked sorghum flour on the alpha amylase digestibility [8]. Elkhalifa at al. detected the increasing effect of fermentation and the decreasing effect of resistant starch on the sorghum starch digestibility [9]. The digestibility of foods (wheat, rice, etc.) was inversely related to the proteins, fats and the total dietary fiber contained in the foods [10].

The main objective of this study is to evaluate the starch digestibility of sorghum and millet grain cereals, grown in the south of Algeria, and to demonstrate the influence of some factors on the kinetics of the hydrolysis.

Our research progressed as follows: first, several samples of sorghum and millet flours were prepared. Then the samples were exposed to different pretreatments and finally the starch digestibility by glucoamylase enzyme was measured.

EXPERIMENTAL

Substrates and enzyme

Sorghum *(Sorghum bicolor (L.) Moench)* and millet (*Pennisetum glaucun*) grains cultivated in Tidikelt, a hyper arid region situated in the south of Algeria, were purchased from the 2005 harvest. All chemical products were Sigma Chemical Co. (St. Louis, MO) and Merck certified grade. The enzyme amyloglucosidase from *Rhizopus Mold* A7255, was Sigma, 23.000 U/g solid of enzyme. Enzyme pepsin was prolabo, 94120, 100 U/mg.

Chemical composition of sorghum and millet flours

We relied on several methods for the estimation of the chemical composition. Ash was measured by NF 03-720 (1981) method described in [11]. Soluble sugars were determined by extraction with alcohol and measured with phenol-sulphuric acid method [12]. Protein content estimation was based on the Kjeldhal method ISI 24-1. The total lipid estimation was based on the method described in [11], ISO 3947. Insoluble fibres estimation was done by Van Soest method [12]. The total humidity was estimated according to ISI 01-1. The total starch content in the flour samples was measured by the enzymatic method [13].

Flour sample preparation (sample 1)

Flour samples from sorghum and millet grains were prepared by milling through a 500 μm screen Retsch AS200 type using a sample mill IKA labotechnik A10.

Sample preparation of protein isolated from sorghum and millet flours (sample 2)

The method used for the pepsin pretreatment of sorghum and millet is given in [14]. Flour samples (equivalent to 2.6 g of starch) were incubated in pepsin solution (0.4 g of enzyme in 100 ml of distilled water) in 250 ml batch reactor. Then 0.1 N hydrochloric acid was added to pH 2.5 for 2 h at 50°C. By the end of the reaction the mixture was filtered and the solid residue was dried in the drying oven at 40°C. The residue was used for the starch digestibility determination.

Sample preparation of lipid isolated from sorghum and millet flours (sample 3)

The method used for a total lipid extraction was described by [15]. Quantity of 2 to 5 g of flour was put in a sample tube used in a Soxhlet extractor of the fat, were shaken for 20 min, each time 10 ml of hexane was added in 35°C. The solid material of the tube was extracted and dried at 40°C overnight to evaporate the solvent.

Sample preparation of protein and lipid isolated from sorghum and millet flours (sample 4)

The protein and lipid were removed by the methods mentioned previously.

Sample preparation of starch isolated (sample 5)

Starch was isolated from the sorghum and millet cultivars by alkali extraction as proposed in [16,17] with few modifications. 10 g of grains were steeped in 20 ml of 0.25 % (w/v) NaOH for 48 h. They were washed and then crushed using a sample mill IKA labotechnik A10. The suspensions were passed through a set of sieves (80, 100 μm). The filtrates were centrifuged (30.000 tour/min during 10 min). The layer of the residual proteins was scraped each time. The extract was then dried at 40°C overnight.

Digestibility test

The enzymatic digestibility of substrats was tested by the method described in [18]. A commercial crystalline glucoamylase enzyme of *Rhizopus mold*, which has high affinity to raw starch, was utilized for the digestion experiments. A reaction mixture consisting 0.13 g equivalent of starch and 6 ml of distilled water in the tubes was placed in a water bath at 100°C for 10 min to obtain the starch suspension. After that the samples were cooled to 40°C for 10 min, then 3.5 ml of 100 mM acetate buffer (pH 5.0) and 3.5 ml of glucoamylase solution (5 unit) were added. The samples were incubated at 40°C for 2 h with stirring. After digestion, surplus starch was removed by centrifuge (4350 tour/min during 10 min), and the contained glucose of the filtrate was analyzed using the glucose oxydase-peroxidase method [19]. The percentage of starch is counted by multiplying the glucose percentage with the factor 0.9. The reported values are the means of triplicate measurements.

Statistical Analyses

Each experiment for digestibility test included three replicates. The difference of means were determined using Student's t-test. Values of $P < 0.05$ were considered statistically significant. The results were evaluated for significance by analysis with SPSS V.13.

RESULTS AND DISCUSSION

Chemical Composition of sorghum and millet flours

Table 1 demonstrates the chemical composition of sorghum and millet flours. The **e**stimated values for the chemical composition of sorghum and millet flours shown in Table 1 are 65 % for total starch, 11-13 % for protein and 4-6 % for lipid. By comparing the results of Barikomo et al. for sorghum and millet cultivated in Mali, the percentage of protein is 10.3 % for sorghum and 7.2 % for millet, the starch content is 65.6 % for millet and 73.5% for sorghum [20]. These results indicate that the proteins and lipids have been affected the starch digestibility of sorghum and millet by glucoamylase.

Digestibility test of samples

The ability of the amyloglucosidase to digest different sorghum and millet flours was studied using different treatments (flours, flours treated by pepsin enzyme, flours treated by hexane, starch, flours treated by pepsin enzyme and hexane solution, raw starch), the

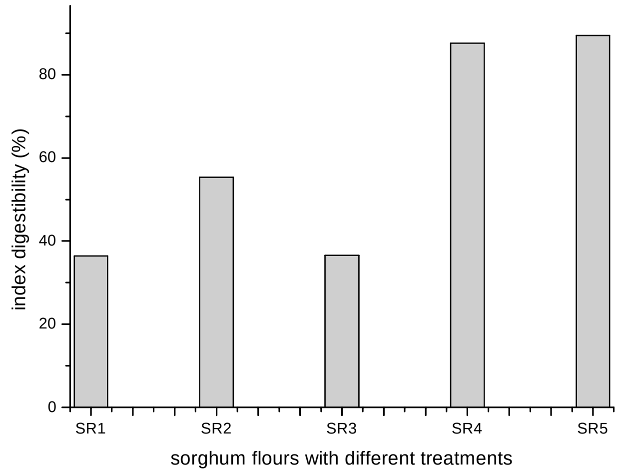

Fig. 1. The index digestibility of sorghum flours at different treatments.

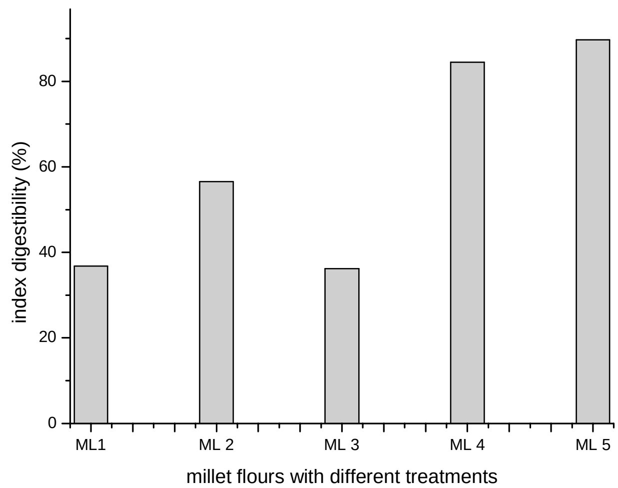

Fig. 2. The index digestibility of millet flours at different treatments.

Table 1**.** Chemical composition of sorghum and millet flours.

Composition, %	Sorghum flour	Millet flour
humidity	9.81	9.37
ash	1.61	2.04
soluble sugar	2.48	3.63
insoluble fibres	5.52	6.64
protein	12.27	11.17
lipid	4.37	6.64
total starch	66.37	65.21

Table 2. The in vitro starch digestibility of sorghum flours at different treatments.

Sample	Starch initial weight, mg	Starch digested weight, mg (a)	Index digestibility, % (b)
SR1	10	3.64±0.10	36.4
SR 2	10	5.54±0.47	55.4
SR 3	10	3.66±0.10	36.6
SR 4	10	8.76±0.29	87.6
SR 5	10	8.95±0.40	89.5

Table 3. The in vitro starch digestibility of millet flours at different treatments.

Sample	Starch·initial·weight,· mg	Starch·digested· weight,·mg	Index·digestibility, %
ML1	10	3.68±0.10	36.8
ML2	10	5.66±0.48	56.6
ML3	10	3.62±0.19	36.2
ML4	10	8.45±0.20	84.5
ML5	10	8.97±0.30	89.7

results are presented in Table 2. Fig. 1 demonstrates in vitro starch digestibility of sorghum starches at different treatments. Table 3 and Fig. 2 present in vitro starch digestibility of millet starches at different treatment.

The digestibility of sorghum flour is 36.4 % and 36.8 % for millet as compared with the results of Snow and O'Dea, the starch hydrolysed of barley flour is 15.06 %, rolled wheat 5.05 % and rye flour 18.63 % [21]. After hydrolysis by amylase and glucoamylase at 50°C for 30 min the digestibility of wheat flour by glucoamylase enzyme is 15 % [18]. This comparison indicates that our sorghum and millet flour are highly hydrolysed in vitro by glucoamylase.

The results indicate that the amyloglucosidase is able to hydrolyse rapidly the starch of sorghum and millet (the sample 5, starch isolation) giving 89.7 % extent of digestibility for millet and 89.5 % for sorghum. The presence of protein in sorghum and millet flours have an impact on the starch digestibility by amyloglucosidase enzyme at different pretreatments, where the digestibility was more increased after removal of some proteins by pepsin (sample 2), 56.6 % for millet and 55.4 % for sorghum, respectively. The low starch digestibility of cooked sorghum flours was studied by Zhang and Hamaker [8].

Effect of the protein and the lipid on the starch digestibility

The influence of the protein and the lipid on the starch digestibility is illustrated in Table 4. According

Table 4. The impact of protein and lipid on sorghum and millet samples.

	Impact of protein (%) (a)	Impact of lipid (%) (b)
sorghum	52.9	33.2
millet	53.3	34.1

(a) impact of protein is the relative digestibility of sample (5) minus the index digestibility of sample (3);
(b) impact of protein is the relative digestibility of sample (5) minus the index digestibility of sample (2).

to the impact of protein and lipid of the sorghum and the millet samples, we notice the that the digestibility is increasing after the removal of some protein and lipid and the impact of protein is 53 % and of lipid is 34 %. These findings indicate that the protein of sorghum and millet plays an important role in slowing the starch digestion.

Generally, we can say that the physico-chemical treatment of sorghum and millet flours plays a role in the industrial processes to convert the starch into dextrines on glucose sugar and influences the hydrolysis of starch by glucoamylase enzyme.

CONCLUSIONS

Based on the results, we deduced that the pretreatments effectuated on sorghum and millet flours has a clear impact on the digestibility. Also, the presence of proteins had a greater impact in lowering the starch digestibility than lipids. Their presence reduces the digestibility as a resistant wall and hindered the passage of the enzyme solution to starch. They increase the susceptibility in the case of isolated starch (sample 5). Besides the starch in sorghum and millet has a great digestibility by glucoamylase (>89 %).

Acknowledgements

We express our gratitude to the laboratory of bioactive products and biomass valorization research. (ENS Kouba, Alger).

REFERENCES

1. P.S. Belton, J.R.N. Taylor, Sorghum and millets: protein source for Africa Trends in food science and technology, **15**, 2004, 94-98.
2. Y. Granfeldt, I. Brorck, A. Drews, An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products, European journal of clinical nutrition., **46**, 9, 1992, 649-660.
3. F. Bornet, Technological treatments of cereals. Repercussions on the physiological properties of starch, Carbohydrate Polymers., **21**,1993, 2-3, 195-203.
4. R. Cui, C. G. Oates, The effect of amylose-lipid complex formation on enzyme susceptibility of sago starch, Food Chemistry, **65**, 1999, 417-425.
5. L. W. Rooney, R. L Pflugfelder, Factors affecting starch digestibility with special emphasis on sorghum and corn, J. Anim. Sci., **63**, 1986, 1607-1623.
6. K. E. Bach Knudsen, L. Munck, B. O. Eggum, Effect of cooking, pH and polyphenol level on carbohydrate composition and nutrition quality of sorghum (Sorghum bicolor (L.) Moench) food, ugali, Br. J. Nutr., **59**, 1988, 31-47.
7. K. E. B. Knudsen, A. W. Kirleis, B. O Eggum, L. Munck, Carbohydrate composition and nutritional quality for rats of sorghum to prepared from decorticated white and whole grain red flour, The journal of nutrition,**118**, 1988, 588-597.
8. G. Zhang, B. R. Hamaker, Low -Amylase Starch Digestibility of Cooked Sorghum Flours and the Effect of Protein, Cereal Chem., 75, 1988, 1, 710-713.
9. A. O. Elkhalifa, B. Schiffler, R. Bernhard, Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour, Nahrung/Food, **48**, 2004, 2, 91-94.
10. A. Urooj, Sh. Puttraj, Digestibility index and factors affecting rate of starch digestion in vitro in conventional food preparation, Nahrung., **43**, 1, 1999, 42 - 47.
11. B. Godon, W. Loisel, Guide Pratique D'Analyse Dans Les Industries de céréales. Technique et Documentation,1984.
12. D.A.T. Southgate, Determination of food carbohydrate, applied science publishers LTD., London,1976.
13. I. Goni, A. Garcia-Alonso, F. Saura-Calixto, A starch hydrolysis procedure to estimate glycemic index, Nutrition Research., **17**, 3, 1997, 427-437.
14. E. T. Mertz, M. M. Hassen, C. Cairns-Whittern, A. W. Kirleis, L. Tu, J. D. Axtell, Pepsin Digestibility of Proteins in Sorghum and Other Major Cereals, Proc, Natl, Acad, Sci., **81**, 1984, 1-2.
15. J. Toivo, A. M. Lampi., S. Aalto, V. Piironen, Factors affecting sample preparation in the gas chromatographic Determination of plant sterols in whole wheat flour, Food Chemistry, 68, 2000, 239-245.
16. T. Beta, H. Corke, L. Rooney, J. R. N. Taylor, Starch properties as affected by sorghum grain chemistry, Journal of the Science of Food and Agriculture, **81**, 2001, 2, 245-251.
17. T. Beta, H. Corke, Genetic and environmental variation in sorghum starch properties, Journal of cereal

science, **34**, 3, 2001, 261-268.
18. T. Noda, S. Takigawa, C. Matsuura-Endoa, K. Saitoa, K. Takata, T. Tabikia, H. Arachichige, M. Wickramasingheb, H. Yamauchia, The physicochemical properties of partially digested starch from sprouted wheat grain, Carbohydrate Polymers, **56**, 2004, 271–277.
19. J.J. Karkalas, An improved enzymatic method for the determination of native and modified starch, Journal of the Science of Food and Agriculture, 36, 1985, 1019–1027.
20. I. Barikmo, F. Ouattara, A. Oshaug, Protein, Carbohydrate and Fibre in cereals from Mali-How to fit the results in a food composition table and database, Journal of food composition and analysis., 17, 2004, 291-300.
21. P. Snow, K. O'Dea, Factors affecting the rate of hydrolysis of in food, the American journal of Clinical Nutrition., 34, 1981, 2721-2727.